





Summary: The impacts of viral infection and subsequent antimicrobials on the microbiome-resistome of growing pigs

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Key Points:

- The fecal microbiome-resistome of growing pigs exhibited a stereotypic trajectory, which shifted rapidly and consistently after weaning and then largely stabilized by 44 days post weaning
- Temporal shifts in the microbiome and resistome far outweighed the impacts of viral challenge and antimicrobial exposures
- PRRSv infection disrupted resistome stability and was associated with an increase in rare yet diverse ARGs

Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease of the US swine production, estimated to cost the industry \$664 million annually. PRRSv infections can increase susceptibility to bacterial infections, and co-infection with multiple bacterial pathogens is common in afflicted pigs. As a consequence of such respiratory co-infections, antimicrobial treatments are crucial for reducing the severity of clinical disease and minimizing morbidity and mortality. While critical for animal health, antimicrobial use can have unintended consequences, including alterations to the microbiome. Antimicrobial use can also have the negative consequence of selecting for antimicrobial resistance (AMR) bacteria within the gut of the exposed pigs. While PRRS is a viral disease, it has been shown to impact the fecal bacterial population of infected pigs, and these impacts seem to be strain- and severity-dependent. These results were based on sequencing and analysis of the 16S rRNA gene and thus were restricted to describing the taxonomic classification of bacteria and archaea. Numerous antimicrobial protocols are used to treat PRRS, but we have little insight into how these treatment schemes impact antimicrobial resistance (AMR) dynamics within the fecal microbiome of commercial swine.

A total of 108 weaned pigs (N=36 per treatment group) were included in the study. Three pigs were placed into each pen, for a total of 12 pens and 36 pigs per treatment group. The 36 pigs in room A ("Minimal" group) were considered the control group. This group was not challenged with PRRSv and did not receive any antimicrobial treatments. The 72 pigs in rooms B and C were challenged with 2×10^3.5 TCID50 of PRRSv 1-7-4 field isolate via the intramuscular route at 44 days post weaning (dpw). At 51 dpw, all 36 pigs in room C ("Intensive" group) were administered 5.0 mg/kg of ceftiofur crystalline free acid (EXCEDE® for Swine, Zoetis) via intramuscular injection. In room B ("Moderate" group), pigs were administered 5.0 mg/kg of ceftiofur crystalline free acid via intramuscular injection if indicated based on clinical signs. Genomic DNA was extracted from pen-level composite fecal samples from each treatment group and subjected to metagenomic sequencing and microbiome-resistome bioinformatic and statistical analysis. Microbiome-resistome profiles were compared over time and between treatment groups.

Fecal microbiome and resistome compositions both changed significantly over time, with a dramatic and stereotypic shift between weaning and 9 days post-weaning (dpw). Antimicrobial resistance gene (ARG) richness and diversity were significantly higher at earlier time points, while microbiome richness and diversity were significantly lower. The post-weaning shift was characterized by transition from a Bacteroides-dominated enterotype to Lactobacillus- and Streptococcus-dominated enterotypes. Both the microbiome and resistome stabilized by 44 dpw, at which point the trajectory of microbiome-resistome maturation began to diverge slightly between the treatment groups, potentially due to physical clustering of the pigs. Challenge with PRRS virus seemed to correspond to the re-appearance of many very rare and low-abundance ARGs within the feces of challenged pigs. Despite very different antimicrobial exposures after challenge with PRRS virus, resistome composition remained largely similar between the treatment groups. Differences in ARG abundance between the groups were mostly driven by temporal changes in abundance that occurred prior to antimicrobial exposures, with the exception of ermG, which increased in the feces of treated pigs, and was significantly more abundant in the feces of these pigs compared to the pigs that did not receive post-PRRS antimicrobials.

The fecal microbiome-resistome of growing pigs exhibited a stereotypic trajectory driven largely by weaning and physiologic aging of the pigs. Events such as viral illness, antimicrobial exposures, and physical grouping of the pigs exerted significant yet relatively minor influence over this trajectory. Therefore, the AMR profile of market-age pigs is the culmination of the life history of the individual pigs and the populations to which they belong. Disease status alone may be a significant driver of AMR in market-age pigs, and understanding the interaction between disease processes and antimicrobial exposures on the swine microbiome-resistome is crucial to developing effective, robust, and reproducible interventions to control AMR.

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